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Detection of Latent Varicella-Zoster Virus Infection in Human Vestibular and Spiral Ganglia

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Varicella-zoster virus (VZV) becomes latent in the sensory ganglia after primary infection and VZV DNA has been found in human trigeminal, thoracic, and geniculate ganglia. In this study, human vestibular and spiral ganglia, which do not receive innervation from the skin, were examined for VZV DNA using the polymerase chain reaction. VZV DNA was detected in 2 of 10 (20%) vestibular ganglia and in 2 of 10 (20%) spiral ganglia from five adults. VZV DNA was undetectable in either type of ganglion from a newborn and from two of the five adults. These two adults were VZV seronegative. The results indicate that VZV becomes latent in several types of sensory ganglion after primary infection and suggest the possibility that reactivation of the virus from the vestibular and spiral ganglia may cause disorders in the labyrinth. ***J. Med. Virol. 51:214–216, 1997.*** © 1997 Wiley-Liss, Inc.

KEY WORDS: polymerase chain reaction; Ramsay Hunt syndrome; vestibular neuronitis; idiopathic sudden hearing loss

INTRODUCTION

Varicella-zoster virus (VZV) becomes latent in the sensory ganglia after primary infection (ckickenpox) and emerges from latency to cause zoster in adults. Besides zoster, reactivation of VZV is known to cause cranial nerve symptoms. Ramsay Hunt syndrome is one of the VZV-associated neurologic diseases with facial paralysis, eighth cranial nerve symptoms, and herpes zoster in the head and neck. The auditory and vestibular symptoms of Ramsay Hunt syndrome are thought to be caused by the spread of inflammation or of the virus to the eighth cranial nerve and the labyrinth. Another possibility exists that reactivation of the virus from the vestibular or spiral ganglia, the sensory ganglia of the eighth cranial nerve, causes the auditory or vestibular dysfunction.

VZV DNA and RNA have been detected in human sensory ganglia from autopsy cases by the nucleic acid hybridization method [Gilden et al., 1983; Hyman et al., 1983; Mahalingam et al., 1990]. Primary and secondary viremia cause the vesicular lesions in the skin. Zoster tends to emerge in the mid-to-lower thoracic, upper lumbar, and ophthalmic dermatomes where chickenpox lesions are concentrated, suggesting that VZV reaches the sensory ganglia by direct neural spread during the primary infection and establishes a latent infection [Hope-Simpson, 1965]. Another possibility is that VZV infects the sensory ganglia during primary and secondary viremia [Meier and Straus, 1992].

The vestibular and spiral ganglia do not have a direct connection with the sensory fibers from the epidermis. VZV latency in the vestibular and spiral ganglia has not been examined yet because these are very small ganglia located in the temporal bone. Previously, we detected VZV DNA in the human geniculate ganglion, which is also a sensory ganglion located in the temporal bone but has a direct connection with the sensory fibers from the skin around the external ear and from the mucosa of the oral cavity [Furuta et al., 1992]. In the present study, we examined human vestibular and spiral ganglia for VZV DNA.

MATERIALS AND METHODS

Ganglia From Autopsy Cases

The temporal bones were dissected from five adults and one newborn at autopsy 1–9 hours after death. The vestibular ganglia were removed from the internal auditory canals. The modioli, which contain the spiral ganglia, were exposed by removing the bony wall of the labyrinth using an electric drill and a microscope. The profile of the cases examined is shown in Table I. None of the patients had symptoms of productive VZV infection (chickenpox or shingles) at the time of autopsy. The ganglia were stored at −70°C until use.

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TABLE I. VZV DNA in Human Vestibular and Spiral Ganglia Obtained at Autopsy

Case no./age (years)	Clinical diagnosis	Vestibular ganglia		Spiral ganglia		VZV antibody
		Right	Left	Right	Left	
1/0	Immature infant	−	NA[a]	−	NA	NA
2/90	Renal cell carcinoma	−	+	−	−	+
3/69	Obstructive ileus	+	−	−	+	+
4/67	Oropharyngeal carcinoma	−	−	−	−	−
5/59	Acute myelocytic leukemia	−	−	+	−	NA
6/43	Acute myocardial infarction	−	−	−	−	−

[a]NA, not available.

Polymerase Chain Reaction (PCR)

The vestibular and spiral ganglia were digested with proteinase K (0.1 μg/ml, Boehringer, Germany) and total DNA was prepared by phenol/chloroform extraction and ethanol precipitation. A pair of primers that are specific for the major DNA-binding protein of VZV (VZV 1, 5′-TACGGGTCTTGCCGGAGCTGGTAT-3′; VZV 2, 5′-AATGCCGTGACCACCAAGTATAAT-3′) was used to detect VZV DNA by PCR [Mahalingam et al., 1990]. PCR amplification was done in a 100 μl reaction mixture consisting of 10 μl of 10× PCR buffer (Perkin Elmer, U.S.A.), deoxynucleotide triphosphate mixture (dATP, dGTP, dCTP, and dTTP at a final concentration of 200 μM), 2.5 units of *Taq* DNA polymerase, 100 pmol of each primer, and 1 μg of sample DNA. Reagents were cycled 30 times on a DNA thermal cycler (Thermal cyclic reactor model TC-100; Hoei Science, Japan), each cycle consisting of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 3 minutes. As an internal standard, reaction mixtures were amplified in parallel with a primer pair specific for the human α-tubulin gene (TUB 1, 5′-GACAGAATTCCAGACCAACC-3′; TUB 2, 5′-GCACCAATCCACAAACGTGA-3′) [Cowan et al., 1983].

Detection of Amplified DNA

One fourth of the final reaction mixture was electrophoresed on agarose gels composed of 1% Seakem and 3% NuSieve (FMC BioProducts, U.S.A.) and was transferred to nylon membranes (Hybond N; Amersham, U.K.). The amplified 273 bp product was detected by Southern blot hybridization and the chemiluminescence method using a digoxigenin-labeled VZV DNA probe generated by PCR, as described previously [Furuta et al., 1992].

VZV Antibody

Serum was taken by cardiac puncture at autopsy and anti-VZV IgG antibody titer was measured using an enzyme immunoassay (SMI Bristol, Japan).

RESULTS

We demonstrated previously the high sensitivity and specificity of the PCR method used in this study [Furuta et al., 1992]. Using PCR with the α-tubulin primers, an amplified DNA fragment of 286 bp was detected in all ganglia. Thus, all samples contained cellular genes that could be amplified. Using PCR with VZV primers, a 273 bp product of VZV DNA was detected in 2 of 10 (20%) vestibular ganglia and in 2 of 10 (20%) spiral ganglia from the five adults (Table I and Fig. 1). Two of the four cases tested were positive for VZV IgG antibody. VZV DNA was detected in the vestibular and/or spiral ganglia from both seropositive cases (cases 2 and 3). In contrast, VZV DNA was undetectable in either type of ganglion from the two seronegative adults (cases 4 and 6) and the newborn (case 1).

DISCUSSION

Mahalingam et al. [1990] reported that VZV DNA was found in 87% of the trigeminal ganglia and in 53% of the thoracic ganglia. Previously, we detected VZV DNA in 79% of the trigeminal ganglia and in 69% of the geniculate ganglia [Furuta et al., 1992]. In the present study, we demonstrated that VZV becomes latent in the vestibular and spiral ganglia after the primary infection. The percentages of VZV DNA positive vestibular and spiral ganglia (20% in each) were lower than those of the other sensory ganglia. VZV is thought to reach the trigeminal, geniculate, and thoracic ganglia by retrograde transport from the skin lesion where VZV replicates at the primary infection (chickenpox). The vestibular and spiral ganglia do not have direct connection with the sensory fibers from the epidermis, however, suggesting that VZV arrives at the sensory ganglia by a hematogenous route during viremia, in which VZV replicates less than in the skin lesion.

It is well known that VZV causes vertigo and/or sudden hearing loss in patients with Ramsay Hunt syndrome. One explanation for the pathogenesis of the eighth cranial nerve symptoms is that latent VZV in the geniculate or trigeminal ganglia reactivates and spreads to the inner ear. Our findings suggest another possibility that reactivation and migration of the virus from the vestibular or spiral ganglion to the labyrinth cause inflammation in the inner ear. In addition, reactivation of VZV in the vestibular or spiral ganglia may cause vestibular neuronitis or idiopathic sudden hearing loss because seroconversions to VZV have been reported in patients with vestibular neuronitis or idiopathic sudden hearing loss [Veltri et al., 1981; Shimizu et al., 1993].

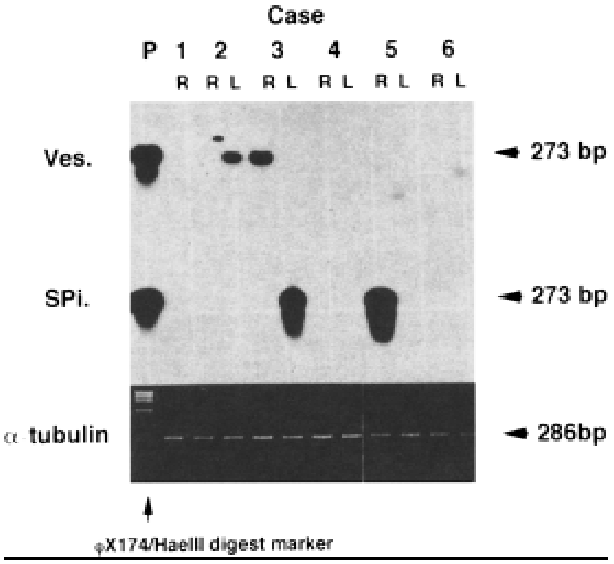

Fig. 1. Detection of VZV DNA in human vestibular (Ves.) and spiral ganglia (Spi.) by PCR amplification and subsequent Southern blot hybridization. P, VZV strain H-N3 DNA (10 fg), used as a positive control; R, right; L, left.

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REFERENCES

Cowan NJ, Dobner PR, Fuchs EV, Cleveland DW (1983): Expression of human α-tubulin genes: Interspecies conservation of 3′ untranslated regions. Molecular and Cellular Biology 3:1738–1745.

Furuta Y, Takasu T, Fukuda S, Sato-Matsumura KC, Inuyama Y, Hondo R, Nagashima K (1992): Detection of varicella-zoster virus DNA in human geniculate ganglia by polymerase chain reaction. Journal of Infectious Diseases 166:1157–1159.

Gilden DH, Vafai A, Shtram Y, Becker Y, Devlin M, Wellish M (1983): Varicella-zoster virus DNA in human sensory ganglia. Nature 306: 478–480.

Hope-Simpson RE (1965): The nature of herpes zoster: A long-term study and a new hypothesis. Proceedings of Royal Society of Medicine 58:9–20.

Hyman RW, Ecker JR, Tenser RB (1983): Varicella-zoster virus RNA in human trigeminal ganglia. Lancet 2:814–816.

Mahalingam R, Wellish M, Wolf W, Dueland AN, Cohrs R, Vafai A, Gilden D (1990): Latent varicella-zoster viral DNA in human trigeminal and thoracic ganglia. New England Journal of Medicine 323:627–631.

Meier JL, Straus SE (1992): Comparative biology of latent varicella-zoster virus and herpes simplex virus infection. Journal of Infectious Diseases 166 (Suppl 1):S13–S23.

Shimizu T, Sekitani T, Hirata T, Hara H (1993): Serum viral antibody titer in vestibular neuronitis. Acta Oto-Laryngologica (Stockholm) Suppl. 503:74–78.

Veltri RW, Wilson WR, Sprinkle PM, Rodman SM, Kavesh DA (1981): The implication of viruses in idiopathic sudden hearing loss: Primary infection or reactivation of latent viruses? Otolaryngology Head and Neck Surgery 89:137–141.